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**EXHIBIT B**

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# EXECUTION

As filed with the Securities and Exchange Commission on July 26, 1993  
Registration No. 33-65328

## SECURITIES AND EXCHANGE COMMISSION Washington, D.C. 20549

### PRE-EFFECTIVE AMENDMENT NO. 1

TO

#### FORM S-1

##### Registration Statement

Under

The Securities Act of 1933

This Registration Statement,  
including exhibits, consists of  
111 pages. Exhibit index appears  
on page 74.

53 18 8315

## TRANSKARYOTIC THERAPIES, INC.

(Exact name of registrant as specified in its charter)

PROCESSED B'

**Delaware**  
State or other jurisdiction of  
incorporation or organization

**2836**  
Primary Standard Industrial  
Classification Code Number

**04-3027191**  
G.R.S. Employer Identification No.: JU 27 1993

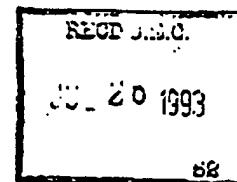
**195 Albany Street  
Cambridge, MA 02139  
(617) 349-0200**

**DISCLOSURE  
INCORPORATE!**

Address, including zip code, and telephone number, including  
area code, of registrant's principal executive offices

**K. Michael Forrest  
President and Chief Executive Officer  
Transkaryotic Therapies, Inc.  
195 Albany Street  
Cambridge, MA 02139  
(617) 349-0200**

Name, address, including zip code, and telephone number,  
including area code, of agent for service



**Leslie H. Shapira, Esq.  
Bingham, Dana & Gould  
150 Federal Street  
Boston, Massachusetts 02110  
(617) 951-8000**

**Mark G. Borden, Esq.  
Hale and Dorr  
60 State Street  
Boston, Massachusetts 02109  
(617) 526-6000**

Approximate date of commencement of proposed sale to the public: As soon as practicable after this  
Registration Statement is declared effective.

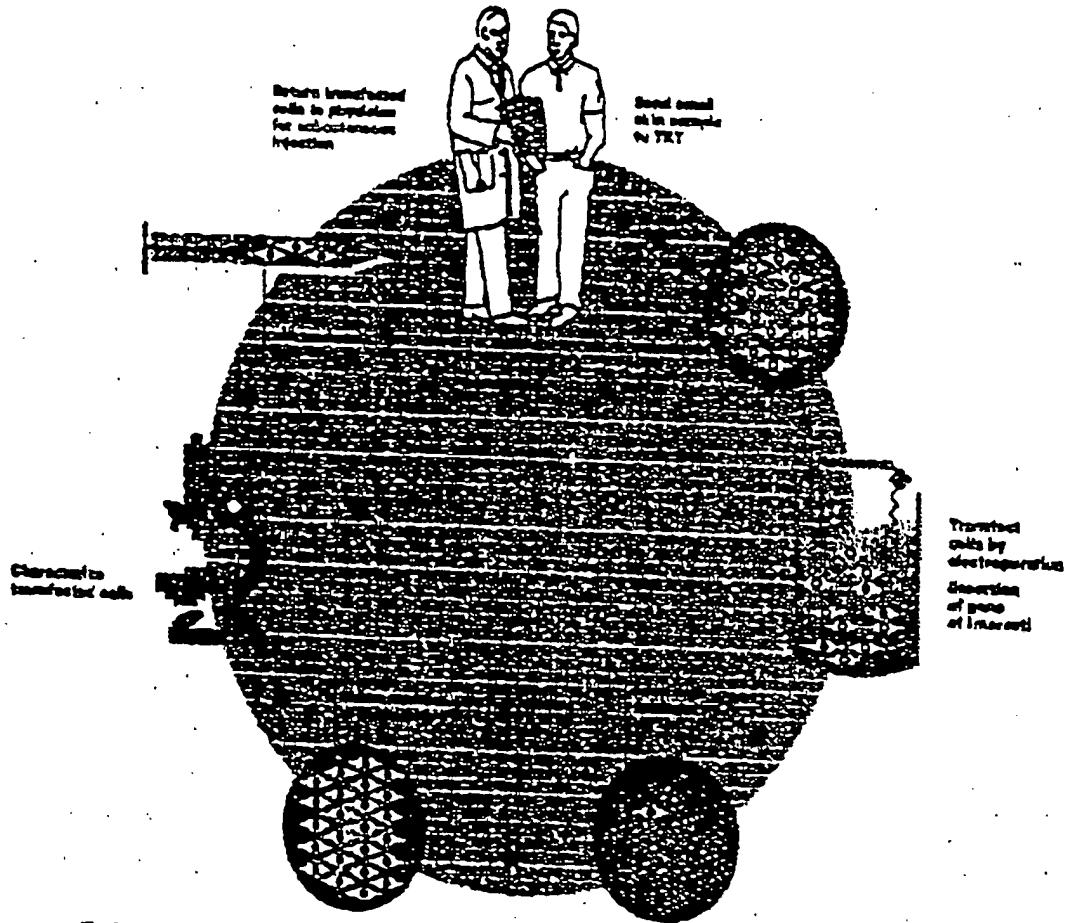
If any of the securities being registered on this form are to be offered on a delayed or continuous basis  
pursuant to Rule 415 under the Securities Act of 1933, check the following box.

The Registrant hereby amends this Registration Statement on such date or dates as may be necessary  
to delay its effective date until the Registrant shall file a further amendment which specifically states that  
this Registration Statement will thereafter become effective in accordance with Section 8(a) of the Securities  
Act of 1933 or until this Registration Statement shall become effective on such date as the Securities and  
Exchange Commission, acting pursuant to said Section 8(a), may determine.

ATTY DOCKET NO.: 7639-017  
SERIAL NO.: 06/102,390  
EXHIBIT: B

### TRANSKARYOTIC THERAPY

TKT's gene therapy system, "Transkaryotic Therapy," is an *in vitro*, non-viral approach to gene therapy. In Transkaryotic Therapy, a patient's cells would be obtained from a small skin biopsy and one or more genes of interest would then be inserted into the cells, a process referred to as "transfection." The transfected cells would be propagated to obtain the desired number of cells and characterized to ensure that they are correctly producing the desired protein. Finally, the genetically engineered cells would be injected under the patient's skin where they would produce the therapeutic protein.



*The Company's product development programs are at an early stage of development and products, if any, resulting from such programs are not expected to be commercially available for a number of years.*

**IN CONNECTION WITH THIS OFFERING, THE UNDERWRITERS MAY OVER-ALLOT OR EFFECT TRANSACTIONS WHICH STABILIZE OR MAINTAIN THE MARKET PRICE OF THE COMMON STOCK OF THE COMPANY AT A LEVEL ABOVE THAT WHICH MIGHT OTHERWISE PREVAIL IN THE OPEN MARKET. SUCH STABILIZING MAY BE EFFECTED IN THE OVER-THE-COUNTER MARKET OR OTHERWISE. SUCH STABILIZING, IF COMMENCED, MAY BE DISCONTINUED AT ANY TIME.**

### PROSPECTUS SUMMARY

The following summary is qualified in its entirety by the more detailed information and financial statements including the notes thereto appearing elsewhere in this Prospectus. The shares of Common Stock offered hereby involve a high degree of risk. See "Risk Factors."

#### The Company

Transkaryotic Therapies, Inc. ("TKT" or the "Company") is dedicated to the development and commercialization of non-viral gene therapy products for the long-term treatment of a broad range of human diseases. Gene therapy is an emerging technology in which genes are inserted into cells to enable synthesis of therapeutic proteins in patients. In the Company's proprietary gene therapy system, "Transkaryotic Therapy," a small sample of the patient's cells would be removed and genetically engineered so that, when implanted in the patient, the cells produce the desired therapeutic protein. In Transkaryotic Therapy, genes are inserted into cells using physical techniques rather than viruses or other infectious agents. The Company is also developing proprietary gene targeting and gene isolation technologies to enhance and support its gene therapy system, as well as to provide independent commercial opportunities.

The Company believes that its gene therapy system may offer several clinical and commercial advantages over conventional treatments and other gene therapies for targeted diseases, including:

- Long-term expression. Transkaryotic Therapy is designed to produce long-term results with a single treatment. In preclinical animal studies, the Company has produced target proteins at levels which the Company believes to be therapeutic for the lifetime of the animals (up to 18 months), suggesting the possibility of long-term effectiveness in humans.
- Safety. Transkaryotic Therapy does not use infectious agents such as retroviruses to genetically engineer the patient's cells. Additionally, TKT's method of producing genetically engineered cells allows for extensive safety testing prior to their implantation in the patient. In studies of TKT's system involving over 3,000 animals, no side effects have been observed.
- Controllability. Transkaryotic Therapy is designed to deliver therapeutic proteins at levels which meet the patient's specific needs. Further, it is anticipated that the treatment afforded by TKT's system will be readily reversible so that therapy can be discontinued if no longer required.
- Ease of administration. Transkaryotic Therapy will allow for the administration of its products by an injection under the patient's skin on an out-patient basis. Furthermore, the potential long-term effectiveness of the treatment could eliminate problems of patient compliance.
- Cost-effectiveness. Transkaryotic Therapy takes advantage of the patient's natural ability to synthesize therapeutic proteins for extended periods. Accordingly, the Company believes that its system may be less costly than conventional protein pharmaceuticals which require frequent administration.

The Company is focusing its initial development efforts on gene therapy products for the delivery of therapeutic proteins for which effectiveness, dose ranges and safety have been clearly established in the context of currently approved and marketed products. The Company's initial products will provide for the delivery of growth hormone for the treatment of cachexia secondary to cancer, Factor VIII and Factor IX for hemophilia A and B, respectively, growth hormone for short stature and erythropoietin ("EPO") for severe anemia. In July 1993, the Company entered into a Collaboration and License Agreement with Genetics Institute, Inc. relating to a joint development and marketing program for a hemophilia A gene therapy product. Based on data generated over the past five years, the Company plans to file an Investigational New Drug application ("IND") in 1993 for its product for the treatment of cachexia secondary to cancer and an IND in 1994 for its product for the treatment of hemophilia B.

The Company's longer-term gene therapy product development strategy is focused on products for the treatment of commonly occurring diseases for which either no protein has yet been proven effective in treating the disease or a proven therapeutic protein exists but effective treatment of the disease requires complex patterns of regulation in the patient. Such long-term products include the delivery of cytokines for the treatment of various cancers, low density lipoprotein ("LDL") receptors for hypercholesterolemia, insulin for diabetes and calcitonin for osteoporosis. A clinical trial at the University of Freiburg, Germany, involving a gene therapy product for the delivery of interleukin-2 for the treatment of renal cell carcinoma, has received regulatory approval in Germany and is expected to commence in 1993. The Company is discussing with the University of Freiburg the terms under which the Company would sponsor these trials and obtain an option to license any product resulting therefrom.

TKT has developed proprietary gene targeting and gene isolation technologies to enhance its gene therapy products. Gene targeting is a technique in which genes are inserted or replaced at a chosen site on a given chromosome. The Company's gene targeting technology is based on attaching a tag to the gene which guides it to the desired location on the target chromosome. TKT's gene targeting technology potentially could improve the overall effectiveness of the Company's gene therapy products and also could allow the development of universal gene therapy products which the Company believes may be suitable for off-the-shelf administration.

Gene isolation is the process of locating a specific gene on a chromosome. TKT's proprietary gene isolation technology is based on a novel approach in which libraries of human DNA are screened in a manner that the Company believes to be more efficient and reliable than existing procedures potentially resulting in a significant reduction in the time, effort and expense required to isolate a desired gene. The Company expects to use its gene isolation technology to seek to discover proprietary genes associated with disease, including diabetes, malignant melanoma and Alzheimer's disease. In July 1992, TKT entered into a three-year research agreement with The Bowman Gray School of Medicine at Wake Forest University under which TKT is sponsoring research seeking the isolation of genes involved in the pathogenesis of diabetes.

The Company has filed six applications for United States patents relating to its gene therapy system, its gene targeting technology and its gene isolation technology. Corresponding foreign patent applications have been filed with respect to certain of these U.S. patent applications. The Company has also entered into licensing agreements under which it has acquired certain worldwide rights to use genes for Factor VIII, Factor IX, carboxylase and LDL receptor in its proprietary non-viral gene therapy products.

#### The Offering

Common Stock offered .....	2,500,000 shares
Common Stock to be outstanding after the offering .....	9,457,780 shares(1)
Use of proceeds .....	Research, preclinical and clinical product development, facilities expansion and other general corporate purposes TKT
NASDAQ Symbol .....	TKT

(1) Excludes 23,780 shares issuable upon exercise of options and warrants outstanding as of July 20, 1991. The number of shares issuable upon conversion of the commanding shares of Class A Preferred Stock, which will vary depending upon the initial public offering price, is based on an assumed initial public offering price of \$10.00 per share. See "Description of Capital Stock—Preferred Stock" and "Capitalization."

### TKT's Technology

#### Transkaryotic Therapy

Transkaryotic Therapy is an *in vitro*, non-viral approach to gene therapy. In TKT's gene therapy system, a patient's cells would be obtained from a small skin biopsy and one or more genes of interest would then be inserted into the cells, a process referred to as "transfection." The transfected cells would then be propagated to obtain the desired number of cells, characterized to ensure that they are correctly producing the desired protein and injected under the patient's skin.

The Company's research and development efforts to date have concentrated on the development of the key components of its gene therapy system:

**Choice of Cells and Propagation.** TKT has worked on a variety of cell types because different cell types may prove appropriate for the treatment of different diseases. TKT is focusing on the isolation and propagation of skin fibroblasts, which TKT believes are particularly well-suited for use in its initial gene therapy products. Fibroblasts, the major cellular component of connective tissue, are present in essentially every organ in the body. Skin fibroblasts are readily accessible by a biopsy, can be efficiently grown in culture, are easily implanted under the skin and can be removed readily following implantation to allow for termination of therapy. The Company has developed proprietary techniques for fibroblast propagation which allow TKT to isolate fibroblasts in large numbers and grow them efficiently and cost-effectively on a large-scale. In its laboratories, the Company has successfully isolated and propagated fibroblasts from over 200 individuals (ranging in age from newborns to 75 years old).

**Insertion of Genes.** TKT has developed a series of proprietary non-viral physical and chemical methodologies for transfection of human fibroblasts. Although it has successfully used other methods to achieve transfection, the Company is focusing on its proprietary method of electroporation, a simple, reproducible and inexpensive method which the Company believes is suitable for large-scale manufacturing. Electroporation involves mixing cells with genes of interest and exposing the cells to an electrical shock lasting a fraction of a second. Following this process, some of the cells take up the genes of interest and incorporate them into their chromosome. Using electroporation techniques developed at TKT, the Company has demonstrated that human fibroblasts can be reproducibly transfected at high efficiency.

In addition to inserting the genes of interest effectively, it is critical that the transfected cells express appropriate levels of the therapeutic protein. A typical human gene consists of two basic components: a promoter region made up of DNA sequences that control the level of expression of the protein and a structural region made up of DNA sequences that encode the recipe for the protein itself. In order to obtain expression of the therapeutic protein, a synthetic fusion of the promoter and structural regions must be precisely engineered using molecular biological techniques. TKT has successfully created such "fusion genes" and has used them to accomplish the expression in fibroblasts of a variety of human proteins, including growth hormone, EPO, Factor VIII, Factor IX, Insulin and the LDL receptor. Having established this ability to express proteins in transfected human fibroblasts, the Company has focused on improving their expression. The Company has engineered fusion genes that have enabled it to improve the expression levels of growth hormone in fibroblasts beyond levels believed by the Company to be required for therapeutic purposes. The techniques that enabled increases in growth hormone expression have subsequently been applied successfully to other proteins and cell types.

**Cell Cloning.** Following transfection, a single cell selected from a pool of transfected cells is cloned (i.e., copied) and propagated, resulting in a uniform population of identical cells for implantation. In many viral systems, genes are randomly inserted at different chromosomal locations in each infected cell. Since the precise location of a gene affects the level of protein expression, the levels of protein expression achieved by many viral systems typically vary from cell to cell. In contrast, the Company clones a cell that is capable of producing the therapeutic protein at desired levels. TKT has cloned and propagated cell populations that produced essentially constant levels of the target protein for the entire *in vitro* life span of the cells. These cloning and propagation techniques have been enhanced and are now routinely and reproducibly performed by TKT. Creating a cloned population of transfected cells produces uniformity in cell function and gene

expression, enabling predictability of expression levels and determination of the optimal number of cells to be implanted.

**Characterization of Transfected Cells.** Prior to implantation into the patient, the clonal population of transfected cells must be characterized to demonstrate that the cells function properly (i.e., they produce the desired amount of the target therapeutic protein) and that they are safe (i.e., they grow normally and produce no toxic substances). The Company believes that this characterization process is facilitated by working with a clonal population rather than the heterogeneous cell populations resulting from viral methods that infect individual cells at random sites. The Company has characterized clonal populations of transfected cells to demonstrate that they produce the target therapeutic protein in the desired amount and that their rate of growth, size and appearance are identical to those of nontransfected fibroblasts in culture.

**Implantation of Transfected Cells.** The Company has investigated a variety of sites suitable for the implantation of transfected cells. In animal studies, TKT has demonstrated that cells may be implanted subcutaneously, intramuscularly, in the abdominal cavity and under the kidney capsule. The Company has chosen to focus on subcutaneous implantation for commercialization because injections under the skin are minimally invasive, well tolerated by patients and generally performed on an out-patient basis.

**Expression of Therapeutic Proteins.** In a series of experiments using over 3,000 animals, TKT has implanted transfected cells producing a variety of therapeutic proteins. For example, in a series of animal experiments involving the implantation of cells transfected to produce growth hormone, the animals expressed growth hormone in the bloodstream at levels that, if achieved in humans, would be therapeutic in patients. Furthermore, growth hormone expression continued for the lifetime of the animals in the study (up to 18 months). No side effects have been observed in any of these animals.

#### **Gene Targeting**

Gene targeting is a technique in which genes are inserted or replaced at a chosen site on a given chromosome. The precise location of a gene affects the level of protein expression. Thus, the Company believes that Transkaryotic Therapy may be further enhanced by the development of its proprietary gene targeting technology.

Gene targeting is based on a naturally occurring process called ~~homologous recombination~~,<sup>12</sup> in which a DNA sequence on one chromosome switches locations with a smaller DNA sequence on another chromosome. The Company's gene targeting technology is based on attaching a tag to the gene which guides it to the desired location on the target chromosome where homologous recombination occurs, resulting in incorporation of the new gene. TKT's gene targeting technology potentially could increase levels of gene expression (suggesting that fewer cells would need to be implanted into the patient) and may eliminate patient-to-patient variations associated with different sites of chromosomal integration.

The Company also believes that its gene targeting technology will permit it to turn on genes that are present in a cell but not normally active in that cell. TKT anticipates that it may be able to use this proprietary technology to activate genes in cells for use either in gene therapy or for manufacturing proteins in commercial quantities.

Finally, the Company is applying its gene targeting technology to the development of cells which would be rendered invisible to the body's immune system through inactivation of the genes that produce certain cell surface proteins that cause the cells to be rejected. Such cells would then be further engineered to deliver therapeutic proteins and thus could potentially serve as "universal" gene therapy products suitable for off-the-shelf administration to any patient with a particular disease.

#### **Gene Isolation**

Gene isolation is the process of locating a specific gene on a chromosome. TKT's proprietary gene isolation technology is based on a novel approach in which libraries of human DNA are screened in a manner that the Company believes to be more efficient and reliable than existing procedures, potentially resulting in a significant reduction in the time, effort and expense required to discover a desired gene. The established technique of isolating and mapping pieces of chromosomal fragments sequentially is known as chromosome walking. The Company's proprietary technique requires only a few hours of a scientist's time for each walking

step, compared to the many weeks of full-time effort generally demanded by conventional methods. Using this technology, the Company has assembled a detailed map of a chromosomal fragment that it believes contains a gene associated with maturity onset diabetes of the young, an inherited form of Type II diabetes. The isolation of this gene could lead to novel therapeutics (including gene therapy) and diagnostics for this disease.

#### TTT's Product Development Programs

TTT's objective is to develop a broad portfolio of products which offer improved treatment of target diseases and conditions, significant healthcare cost savings and better quality of life to patients. The Company's gene therapy product development programs seek to develop patient-specific products for the treatment of hematologic disorders, endocrine/metabolic disorders, cancer and cardiovascular disease.

The Company also intends to develop additional products utilizing its gene targeting and gene isolation technologies. TTT is currently using its gene targeting technology to develop universal gene therapy products suitable for off-the-shelf administration. The Company's gene isolation program is currently focused on discovering proprietary genes associated with disease, including diabetes, malignant melanoma and Alzheimer's disease.

The following chart summarizes the status of each of the Company's current gene therapy product development programs.

<u>Indication</u>	<u>Protein To Be Delivered</u>	<u>Development Status(1)</u>
<b>Initial Products</b>		
Cachexia	Growth hormone	Precinical; IND to be filed in 1993
Hemophilia B	Factor IX	Precinical; IND to be filed in 1994
Hemophilia A	Factor VII	Research
Short stature	Growth hormone	Precinical
Severe anemia	Erythropoietin	Precinical
<b>Longer-Term Products</b>		
Renal cell carcinoma	Interleukin-2	CTX approved
Hypercholesterolemia	LDL receptor	Research
Diabetes	Insulin	Research
Osteoporosis	Calcitonin	Research

(1) "Research" indicates that work is being conducted to identify lead compounds, including the construction of fusion genes and the testing of such genes in human cells.  
 "Precinical" indicates that pharmacology, toxicology and efficacy testing in model systems (*in vitro* and animal) is being conducted to gather information on physiologic regulation and/or data necessary to comply with applicable regulations prior to commencement of human clinical trials. All dates for IND filings are estimated.  
 "CTX approved" indicates that a clinical trial exemption certificate has been granted by German regulatory authorities to a principal investigator for permission to begin clinical trials. Subject to execution of definitive documentation, the Company expects to sponsor these trials. See "Business—TTT's Product Development Programs—Longer-Term Gene Therapy Products—Renal Cell Carcinoma."

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